Expert Opinion

informa
healthcare

Non-viral gene delivery using nanoparticles

Andrew J Ditto, Parth N Shah & Yang H Yun†
†*The University of Akron, Sidney Olson Research Center, Department of Biomedical Engineering, Akron, OH 44325, USA*

Although the potential benefits of gene therapy for the treatment of acquired and inherited genetic diseases have been demonstrated through preclinical studies, the results of human gene therapy trials have been disappointing. Recombinant viruses are the primary vectors of choice because of their ability to protect genetic materials, cross cellular membranes, escape from endosomes and transport their genetic materials into the nucleus. Unfortunately, viral vectors have been unable to gain widespread clinical application because of their toxicity and immunogenicity. Consequently, the need for safer alternatives has led to the development of liposomes, cationic polyplexes, microparticles and nanoparticles. Although these alternative vectors have shown promise, degradable nanoparticles are the only non-viral vectors that can provide a targeted intracellular delivery with controlled release properties. Furthermore, the potential advantage of degradable nanoparticles over their non-degradable counterparts is the reduced toxicity and the avoidance of accumulation within the target tissue after repeated administration. In this article, current non-viral gene delivery devices are reviewed with a special emphasis on nanoparticle gene delivery systems. Also, the authors highlight their philosophy and efforts on the development of L-tyrosine-based polyphosphate nanoparticle-based non-viral gene delivery systems and assess the potential benefits and shortcomings of their approach.

Keywords: controlled release, gene therapy, nanoparticles, non-viral

Expert Opin. Drug Deliv. (2009) 6(11):1149-1160

1. Introduction

Gene therapy is a promising approach for the treatment of an assortment of diseases. Although a wide variety of vectors designed to deliver therapeutic genes have been investigated, most efforts have focused on viral vectors because of their ability to recognize cells, traverse across the cellular membrane, escape from endosomes, and transport their genetic material to the nucleus. As a result, most viral vectors achieve a high degree of protein expression and are very attractive for applications in clinical therapies. Despite these benefits, the research into non-viral gene delivery vectors has accelerated in the recent past. The primary goal has been to emulate the desirable characteristics of viral vectors while overcoming problems that are inherent with these delivery systems, such as their immunogenicity and toxicity reactions, the potential for catastrophic viral recombination, their limited payload capacity, and large-scale production issues [1].

The unique properties of nanoscale matter, the diversity of available materials, and countless design formulations have all contributed to the emergence of non-viral vectors [2]. These include cationic polyplexes, lipoplexes, liposomes as well as biodegradable and non-degradable nanoparticles. Ideally, a non-viral gene delivery vector should be capable of transporting the encapsulated genetic material to the desired site, interacting with the intended tissue or cell type, and delivering the genetic payload into the cells and possibly into the nucleus without showing

10.1517/17425240903241796 © 2009 Informa UK Ltd ISSN 1742-5247

toxicity or eliciting an immune response. Also, a direct interaction with cellular machinery would definitely be advantageous for all gene delivery systems. Therefore, delivery vehicles must have the appropriate dimensions for endocytosis. Finally, a rapid degradation rate is also desirable for intracellular delivery so that the encapsulated genetic material can be released within the lifetime of most cells, achieve optimum efficiency, and prevent the accumulation of the delivery vehicle in the tissues.

No single gene delivery vehicle contains all of the above-mentioned desirable characteristics at present, and each has its advantages and disadvantages. However, all these desired properties exist separately. As nanoparticles are discrete and can encapsulate a variety of materials, they are an ideal platform for the incorporation of all these sought-after characteristics into a single system. Nanoparticles should be fabricated from rapidly degrading polymers, so their release of genetic material is within the cell's lifetime and thereby achieves optimum efficiency. The incorporation of cationic polymers, nuclear localization signals, and poly(ethylene glycol) (PEG) into these nanoparticles provides them with the ability to escape endosomes, navigate to the nucleus, and evade the immune system. In addition, the surfaces of nanoparticles can be decorated with peptides or antibodies for cellular targeting. Once all of these viral-like components have been incorporated into nanoparticles, non-viral gene therapy may become a viable clinical option. Therefore, L-tyrosine polyphosphate (LTP), a rapidly degrading and non-cytotoxic polymer, has been adapted as a platform for formulating gene delivery systems [3]. So far, several components have been incorporated into LTP nanoparticles that mimic critical viral functions that are responsible for their transfection efficiency in order to overcome natural cellular barriers (**Figure 1**) [4]. If formulated appropriately, the authors believe LTP nanoparticles may be a viable option for clinical gene therapy [3,4].

1.1 Viral vectors

Viruses are nature's nanotechnology. They are efficient in recognizing and invading the host cells and seizing their protein synthesis mechanism for self-replication. For gene therapy, the use of recombinant viral vectors is very attractive because their cellular targeting and the efficiency of transfection remain intact while the unwanted viral genes are replaced with therapeutic sequences. For these reasons, most of the gene therapy clinical trials approved by the National Institutes of Health (NIH) have used recombinant viral vectors [5].

Although the possibility of developing infectious diseases from intact viruses has been considerably lowered, the immune response to their protein coat still remains and has raised safety issues [6]. These concerns come from fatalities reported in gene therapy trials and from the potential for the induction of cancer [7]. In 2000, Jesse Gelsinger died after receiving an adenovirus vector for the treatment of ornithine transcarbamylase deficiency [8]. Seven years later, a 36-year-old mother died after injections of an adeno-associated virus for the treatment of rheumatoid arthritis [9]. A recent study published in *Nature* has linked the treatment of X-linked severe combined immunodeficiency (X-SCID) using retroviral vectors, the only known cure using gene therapy, to an unforeseen and unintended elevated risk of developing leukemia [10]. These unfortunate incidents underscore the necessity for safer vectors that can mimic a virus'- natural ability to deliver genes without the associated problems of their protein coats.

1.2 Non-viral vectors

Although viral vectors have distinct advantages over plasmid DNA (pDNA), especially in transfection efficiencies, they can be toxic, immunogenic, potentially activate oncogenes, and/or deactivate tumor suppressor genes. To circumvent these issues, the use of pDNA encoding for therapeutic genes is highly desirable and could be used to achieve the same clinical goals as viral therapies. However, the application of naked pDNA without a carrier is not an efficient method for gene therapy administration.

Designing a gene delivery vector that is safe to patients and achieving the optimal expression of therapeutic protein are highly desirable goals that remain elusive. Whereas safety is the central concern in the design of an effective vector for gene delivery, the issue of optimal delivery has been only partially addressed. Utilization of pDNA appears to fulfill some of the requirements for gene delivery. However, the lack of proper mechanisms for controlling pDNA delivery and localization to the targeted cells could easily result in poor therapeutic outcomes. Further complicating the situation is that the surface charge of the cells repels DNA, blood flow can carry the DNA away from the intended tissue shortly after administration, and DNA itself is not protected from degradation by nucleases. Therefore, an effective non-viral gene delivery system is needed.

2. The application of nanotechnology for gene delivery

Innovations in nanotechnology in recent years have generated a considerable interest for medical applications. The NIH defines nanotechnology as the understanding and control of matter at dimensions of 1 – 100 nm. However, others have expanded this definition to encompass larger objects up to 1000 nm [11,12]. Nanotechnology could provide the means for directly accessing and manipulating the internal cellular machinery, such as protein transcription and translation, DNA replication, and RNA silencing [13,14]. The introduction of genetic materials using nanotechnology could provide a practicable alternative to the viral delivery systems without the associated immune response. For these systems to be effective, the delivery vehicles must have appropriate dimensions for endocytosis [15], rapid degradation rates [16], and be non-toxic [17].

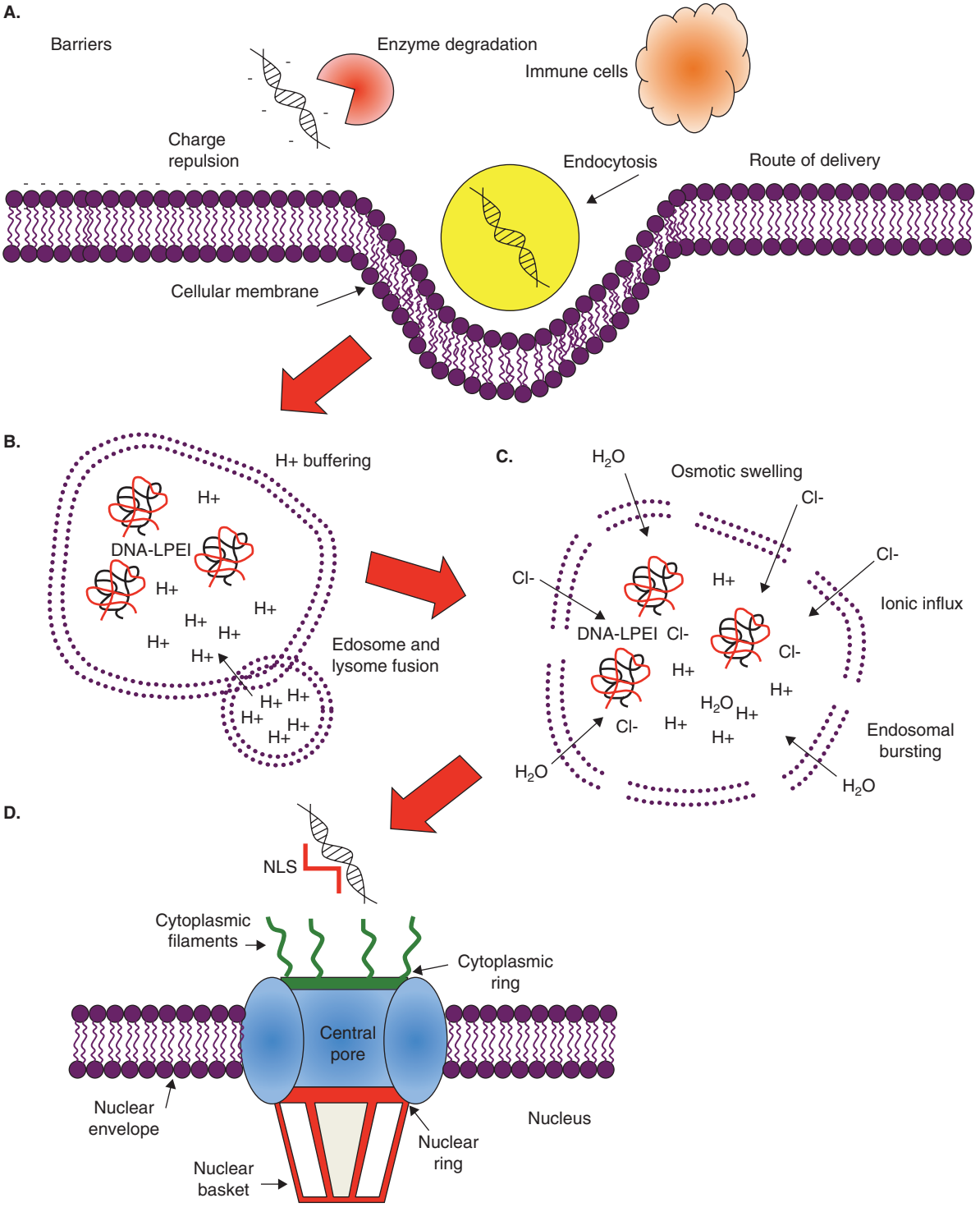

Figure 1. Barriers to gene delivery that include: degrading enzymes, the immune system, membrane charge repulsion and endosomal entrapment. Routes of delivery that include: **(A)** endocytosis; **(B, C)** escape from endosomes; and **(D)** passage through nuclear pores.
NLS: Nuclear location signal.

2.1 Lipoplexes and cationic polyplexes

Cationic lipoplexes and polyplexes are nanoscale complexes obtained by the self-assembly of cationic lipids or polymers with nucleic acids, which neutralize the charge and allow for the passage of these complexes across cellular membranes [18]. A wide array of polycations is available for complex formation, including those with linear, branched, dendritic, and block as well as graft copolymer architectures [19]. Similarly, cationic lipids with varying architectures are also available [20]. These lipid and polymer-based complexes are 50 – 100 nm in diameter, can neutralize the negative charge of the DNA, interact with cellular membranes, and enter into the cytoplasm through various mechanisms [21]. Studies have shown that smaller complexes have a higher transfection efficiency and lower toxicity than larger complexes. For example, linear polyethylenimine (LPEI, 25 KDa) has a higher transfection efficiency and lower toxicity than branched polyethylenimine (BPEI) [22,23]. The size of these complexes can be controlled primarily by varying the ionic strengths and concentrations of the solutions in which they are formed. Generally, the transfection efficiency for both polyplexes and lipoplexes is a function of various parameters, including physicochemical characteristics of the polymers, complex size, morphology, net charge, mode of complex formation, and so on [24]. Although reasonably favorable results in terms of transfection efficiencies have been obtained for systems based on higher molecular mass components under both *in vitro* as well as *in vivo* conditions, most of these systems are plagued by a moderate-to-high degree of cytotoxicity, which suggests cellular membrane disruption and lysis [25]. For example, cationic polyplexes based on polymers, such as poly(lysine) (PLL), LPEI and BPEI, can carry large amounts of pDNA; however, they are toxic at high dosages and are easily cleared from the circulation [26]. Furthermore, current literature strongly indicates that both lipid-based and homopolymer-based complex systems show significant limitations pertaining to pharmacokinetics and biodistribution under *in vivo* conditions [27]. These problems have been attributed to the nonspecific electrostatic interactions between the complexes and tissues [19]. Finally, the gene expression efficiencies of cationic lipoplexes and polyplexes are still relatively low when compared with viruses and achieve only a brief, transient transfection for 24 – 72 h [28]. Thus, a less toxic and sustainable gene delivery system would enhance the overall gene expression and cellular viability.

Efforts to resolve some of these problems have been made by adopting variations of the commonly used cationic polymers for the formulation of DNA polyplexes. Linking cationic copolymers with a non-ionic water-soluble polymer, such as polyethylene oxide (PEO), is a common strategy. Examples include PEO-*b*-polyspermine, PEO-*b*-polylysine, PEO-*g*-polylysine, and PEO-*g*-polyethyleneimine [29-31]. Unfortunately, these block polymers show very limited activity when compared with the unmodified polycations. Thus, their efficacies have been compromised by efforts to increase the biocompatibility.

2.2 Liposomes

Liposomes consist of a lipid-based bilayer membrane surrounding an inner core for hydrophilic drugs, which, unlike lipoplexes and polyplexes, can encapsulate genetic materials through self-assembly [32,33]. As lipids self-assemble into a discrete structure, the surfaces of the liposomes can be decorated with PEG, targeting moieties, and other desired functionalities by incorporating amphiphilic molecules. Thus, liposomes can be modified with viral-like components for enhanced efficacies over lipoplexes or polyplexes. However, the toxicity associated with liposomes is linked to their method of gaining entry into the cell and has been their main drawback [34,35]. As they fuse with the plasma membrane, liposomes have the potential for cellular lyses and the controlled release of DNA cannot be attained. Other problems associated with liposomes include poor loading efficiencies, a loss of bioactivity of the genetic material during fabrication, short half-lifes in circulation, and low transfections [36]. However, variants of liposomes, such as pH-sensitive liposomes and immunoliposomes, have been proposed to improve the gene expression efficiencies [37]. In general, a less toxic and sustainable gene delivery system would enhance the overall gene expression and cellular viability.

2.3 Nanoparticles

Nanoparticles are ideal candidates for intracellular gene vectors because they can be tailored for a sustained delivery of genes, and their sizes generally vary from 10 to 1000 nm, which means eukaryotic cells can internalize them [38,39]. As with liposomes, the nanoparticle's surface can be easily adapted for cellular targeting. The addition of other functionalities that mimic viral behavior can be easily incorporated. An ideal nanoparticle must possess the following essential criteria as a gene vector: the ability to recognize cells through targeting, to traverse across the cellular membrane preferably by endocytosis, to escape from endosomes, to provide a sustained intracellular release to increase the gene's bioavailability before being cleared from the body, and to transport its genetic material to the nucleus (**Figure 1**) [40-42].

3. Materials for nanoparticle formulation

Numerous materials from both inorganic and organic sources have been investigated for the formulation of nanoparticles for gene delivery applications (**Table 1**). Inorganic nanoparticles possess several attractive features, such as wide availability, rich surface functionality, ease of functionalization, good biocompatibility, and potential for site-specific delivery. Therefore, these devices have generated sizeable attention, and a large number of inorganic nanoparticles have been adapted for gene delivery applications. The most common materials for inorganic nanoparticle formulations include fullerenes, carbon nanotubes, layered double hydroxides, silicon oxides, paramagnetic iron oxides, calcium phosphates, and metals such as gold and silver. Although inorganic nanoparticle formulations have

Table 1. Common materials used to prepare biodegradable nanoparticles for gene delivery.

Material	Degradation period	Formation method	Advantages	Disadvantages
LTP [3,4]	1 week	Single emulsion	Rapid degradation, low toxicity	Lack of data
Alginate [18]	1 month	Single emulsion	Low toxicity	Slow degradation
PLGA [30,33]	1 – 2 months	Double emulsion	Versatility in processing	Slow degradation, Acidic products
PCL [63,65]	> 2 months	Double emulsion	Versatility in processing	Slow degradation
PLA [44]	> 2 months	Double emulsion	Versatility in processing	Slow degradation, Acidic products
Hyaluronan [64]	> 2 months	Single emulsion	Low toxicity	Slow degradation, expensive
Chitosan [18]	> 2 months	Single emulsion	Low toxicity	Slow degradation
PEG [52]	> 2 months	Single emulsion	Decreased protein absorption	Slow degradation, No clearance by the kidneys with M_w > 6 kDa

several advantages, the primary mechanism to load/carry and deliver genetic material is through the use of electrostatic, hydrophobic, and hydrophilic forces. Therefore, surface modification is a central theme for the preparation of all inorganic nanoparticle formulations, and the loading is generally limited by surface interactions and the availability of the particle's surface for interactions. The release of DNA is always passive with limited flexibility. The transfection efficiency in most cases has been noted to be relatively low [43]. Although most inorganic nanoparticles have been deemed to be biocompatible and possess low cytotoxicity, all inorganic nanoparticles, with the exception of those formulated using layered double hydroxides, are unable to degrade under clinically relevant conditions [43]. A major implication of this finding is that repeated dosing results in the accumulation of inorganic nanoparticles within the cells following endocytosis, which may lead to a higher cytotoxicity than previously believed.

As a practical alternative to inorganic materials, a wide variety of natural and synthetic polymeric biomaterials have been investigated for the development of nanoparticles (**Table 1**). Despite the myriad of available materials, only a few meet the requirements for a biomedical device. Natural biodegradable polymers, such as collagen, gelatin, hemoglobin, serum albumin, alginate, and hyaluronan, have been studied for controlled drug delivery device development [44]. The use of these polymers is limited by methods of fabrication, and these materials have lot-to-lot variations [45]. Furthermore, proteins can be easily denatured by temperature, physical forces, and solvents.

3.1 Synthetic polymers

By contrast, the application of synthetic biomaterials for drug delivery and implantable devices has escalated, especially in the last two decades, because they are free from most of the problems associated with natural polymers [45-47]. However, all polymers designed for delivery systems must meet several requirements, which include biocompatibility, drug compatibility, suitable biodegradation kinetics, mechanical properties, ease of processing, and availability of the material [48,49]. A degradable polymeric nanoparticle is advantageous because the drug release can be tailored by the use of various polymers that can degrade at different rates through hydrolytic, enzymatic, oxidative, or a combination of pathways.

Commonly used degradable polymers that degrade under physiological conditions include poly(lactic-*co*-glycolic acid) (PLGA), poly(caprolactone) (PCL), poly(acrylic acid) (PAA), and so on. Unfortunately, nanoparticles derived from most polymers lack the appropriate degradation rate for intracellular delivery [50-52]. For example, PLGA and PCL can take months to degrade and release their encapsulated drugs or genes [53-55]. Although PLGA and PCL's release rates may be suitable for applications with depot delivery, these degradation rates exceed the lifetime of most cells and cause the nanoparticles to persist in the tissues for extended periods. Also, studies have shown that the degradation products of polyesters, such as PCL and PLGA, can lead to a localized drop in pH [56,57], and the persistence of artificial materials in the tissues has been shown to be cytotoxic, to induce inflammation, and to initiate the immune response [58].

3.2 L-tyrosine polyphosphate

LTP, a polymer developed from an amino acid, is an ideal candidate for designing a gene delivery system [3,4,56,59]. Amino acids are the building blocks of proteins that are polymerized using peptide bonds and are folded into secondary and tertiary structures with ease by cells. Unfortunately, this technique offers limited options for preparing drug delivery devices, such as nanoparticles. In general, poly(amino acids) such as poly(L-tyrosine) have found limited applications in biomedical devices because of problems associated with their synthesis and processing.

The limitations associated with the processing of poly(amino acids) have been overcome by chemical modification of the structure of poly(amino acids) through the incorporation of alternating non-peptide functionality along with the peptide bonds within the backbone (**Figure 2**).

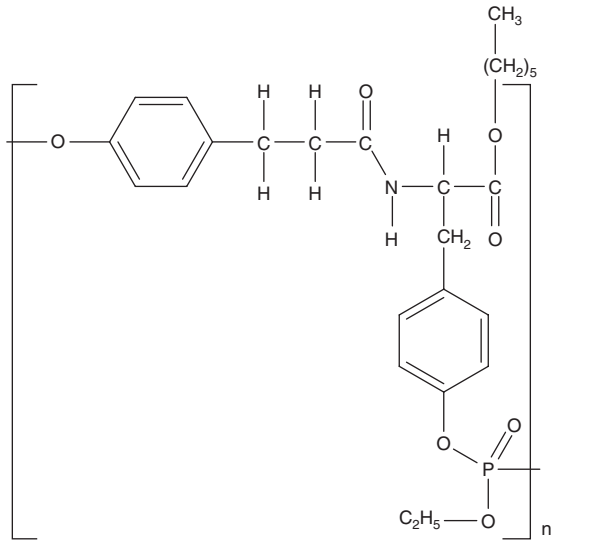

Figure 2. Chemical structure of L-tyrosine polyphosphate [3].
Reproduced with permission.

Specifically, the structure of L-tyrosine has been modified with two coupling agents. Desaminotyrosine, a molecule that resembles the structure of L-DOPA, is linked to the amine group of L-tyrosine through a peptide bond. Since a polymer with a rapid biodegradation is advantageous for gene delivery applications using nanoparticles, a phosphate is coupled to the terminal hydroxyl group of the amino acid. The polymerization of repeating units results in LTP with a molecular mass of 8 – 10 kDa [56,59], and the resulting polymer is classified as a 'pseudo' poly(amino acid). LTP is soluble in common organic solvents such as methylene chloride and chloroform [56,59]; thereby, it is easily processed into a nanoparticle formulation using traditional emulsion methods.

Unlike most biodegradable polymers, LTP can degrade in a 7-day period, which is in an appropriate time frame for intracellular delivery. This rapid degradation gives nanoparticles formulated from LTP a distinct advantage over other biodegradable nanoparticles as a gene vector. Previous studies show that LTP nanoparticles loaded with pDNA also degrade within 7 – 10 days because of the presence of hydrolytically unstable phosphoester linkages in the LTP's polymeric backbone [3,60]. These results agree with previous degradation studies with LTP films [56,59]. As desaminotyrosine is coupled to L-tyrosine by a peptide bond, our nanoparticles can be degraded by an enzymatic pathway as well as hydrolysis. The degradation products, which are desaminotyrosine, L-tyrosine, phosphates and alcohols, have been shown to be non-toxic [60] and to have a negligible effect on local pH [56]. The lack of acidic and toxic degradation products shown by LTP is unlike PLGA and PCL. Finally, LTP nanoparticles have been shown to be non-cytotoxic to human fibroblasts [3,4,60], which also have the ability to internalize these nanoparticles [3].

3.3 LTP nanoparticles and the role of amphiphilic polymers

A one-step emulsion-based process has been developed for the formulation of LTP nanoparticles. Poly(ethylene glycol) grafted onto chitosan (PEG-*g*-CHN) has several functions in our nanoparticle formulations. As PEG-*g*-CHN is amphiphilic, it will accumulate at the water and oil interface and help stabilize the oil-in-water emulsion. PEG also has been recognized as a 'stealth molecule' because it resists protein adsorption and cellular interactions [61,62]. Thus, the incorporation of PEG-*g*-CHN into an oil-in-water emulsion will form a steric layer (i.e., PEGylated surface) on the nanoparticle's surface [62,63]. As proteins and cells interact only at the biomaterial surfaces, the accumulation of PEG at the oil–water interface will increase the nanoparticles' circulation life in plasma and impart favorable biocompatibility. Studies have found that the incorporation of PEG into nanoparticle formulations results in extended circulation in the bloodstream and reduced liver uptake when compared with unmodified nanoparticles after intravenous injections [64,65]. The PEG used to synthesize the PEG-*g*-CHN has a molecular mass of 2 kDa, thus it can be excreted under *in vivo* conditions once the PEG-*g*-CHN degrades [66]. Finally, PEG can be functionalized and adapted for the conjugation of targeting moieties.

LPEI is a cationic polymer that is also needed to help stabilize the primary emulsion of the LTP nanoparticles. However, the added benefits of LPEI include the abilities to protect DNA during sonication [60], to buffer the pH of lysosomes, and to induce an osmotic pressure gradient that causes swelling and bursting of endosomes [18]. This process is also known as the 'proton-sponge' theory. Thus, LPEI released from nanoparticles interferes with lysosomal activities and provides LTP nanoparticles with a method of escape from vesicle entrapment. Afterwards, LTP nanoparticles can diffuse into the cytoplasm where they can degrade further and release pDNA at a controlled rate.

4. Nanoparticle targeting

The ability to target specific cells is essential for therapeutic efficiency as well as for the safety of the patient. The incorporation of targeting moieties that can recognize a specific cell type will enhance the efficacy of the nanoparticles, improve the bioavailability of the therapeutic agent at the desired site, and obviate unwanted effects in non-target cells. Targeting also allows for systemic delivery, which allows for a relatively simple means of gene administration.

At present, the most common means of targeting a specific cell type for gene therapy is through the incorporation of peptides onto the nanoparticles' surface (**Table 2**). For example, researchers have used the arginine-glycine-aspartic acid (RGD) peptide from fibronectin to target integrins expressed on the surface of vascular endothelial cells [67]. These peptides can be blended into the nanoparticles during their formulation,

Table 2. Selected examples of targeting groups for nanoparticles used in gene therapy.

Targeting group	Targeting type	Efficacy and description
Streptavidin [14]	Peptide	Targeted fibroblasts with a 20-fold increase of transfection efficiency compared with nanoparticles without streptavidin conjugate
E and P selectin [64]	Monoclonal antibody	Targeted CHO-EP cells with a mean of 80 nanoparticles/mm^2 compared with 15 nanoparticles/mm^2 for CHO cells
Folate [25]	Small molecular mass molecule	Targeted KB cells with a threefold increase of transfection efficiency compared with liposomes without folate group
Integrin receptor-targeting domain [71]	Peptide	Targeted tracheal epithelial cells with a 10-fold increase in transfection efficiency compared with nanoparticles without integrin receptor-targeting domain
αvβ3/αvβ5 integrin-binding RGD peptide [90]	Peptide	Target angiogenic endothelial cells with a fivefold increase in transfection efficiency compared with nanoparticles without peptide

chemically bound to the materials used or adsorbed onto the particle surface following device formulation. Recently, researchers have explored the use of functionalized PEG grafted to a polymer as an avenue for targeting nanoparticles. Examples include functionalized PEG-*g*-polylysine, functionalized PEG-*g*-PEI, and functionalized PEG-*g*-PLGA. Targeting moieties can then be conjugated to the functionalized PEG and be easily incorporated into the nanoparticles during the emulsion formulation. To achieve targeting for the LTP nanoparticles, a folate molecule was conjugated to functionalized PEG-*g*-PLGA (**Table 2**). Nanoparticles were then decorated with folate molecules by direct substitution with PEG-*g*-CHN by mass. The efficacy of targeting has been tested using a parallel flow chamber and a cancerous cell line that overexpresses the folate receptor. Under physiological flowing conditions of 0.5 dyn/cm^2, the authors' results show 127.7 ± 8.0 nanoparticles/mm^2 adhered to HeLa cells. By contrast, LTP nanoparticles without the targeting moiety show little adhesion (17 ± 2.0 nanoparticles/mm^2). This initial study confirms the proof of concept that LTP nanoparticles can be modified to target and to bind specific cells under a flowing condition.

The use of whole antibodies and antibody fragments has also been explored for the targeted delivery of genes (**Table 2**). This method of targeting is usually accomplished by adsorbing protein A onto the surface of the nanoparticles and then conjugating antibodies to the protein A. The nonspecific regions of the antibodies react with protein A in a site-specific manner so that cellular recognition sequences are directed outwards from the nanoparticles to the cells. Microparticles and nanoparticles formulated from various polymers, such as hyaluronan, PCL and PLGA, have been successfully adapted for cellular targeting using this method [68,69].

5. Nanoparticle trafficking

Similar to viruses, the primary entry route for nanoparticles into the cell is by receptor-mediated endocytosis, which is followed by endosomal encapsulation [70]. However, various endocytic pathways exist for nanoparticles to gain entry into cells, such as clathrin- and caveolin-mediated endocytosis, macropinocytosis, and phagocytosis. Recent evidence suggests that the specific endocytic process participating in the nanoparticle uptake is significant because it may influence intracellular trafficking and ultimately transfection [71,72]. The subsequent internalization and trafficking pathways depend on the cell type as well as the size, charge, composition, and stability of the nanoparticles [73].

5.1 Endocytosis

It has been demonstrated that particles < 1 μm utilize both clathrin-dependent and clathrin- and caveolin-independent endocytosis, in which the cellular membrane invaginates and pinches off to form vesicles around the particles [70]. These vesicles are quickly uncoated and become early endosomes. Typically, these early endosomes are directed to sorting endosomes, where the nanoparticles are either transported to the surface and exocytosed or trafficked to the lysosomal pathway [70]. Once the late endosome is destined for the lysosomal pathway, the compartments undergo gradual acidification as they merge with lysosomes. After the lysosome merges with the late endosome, degradation occurs with an influx of protons and various enzymes. On the other hand, larger particles > 1 μm are internalized by caveolin-dependent endocytosis, macropinocytosis, or phagocytosis, which results in nanoparticles entrapped in caveosomes, macopinosomes, or phagaosomes. These compartments lack certain signaling molecules and do not advance to the endolysosomal pathway, which results in the bypass of acidic degradation [74]. For the vectors that are not transferred to the endolysosomal pathway have a greater chance of being exocytosed [75], trafficking from early endosomes to late endosomes and to the lysosomal pathway is critical for both the nuclear translocation and subsequent transfection when using nonviral vectors. Several studies, including research by Patil *et al.*, have shown that endocytosed degradable PLGA nanoparticles ranging from 100 to 1000 nm are taken up by the endolysosomal pathway [76]. Furthermore, Patil *et al.* have

Table 3. Materials for DNA condensation.

Materials	Minimum diameter size
Chitosan	75 ± 25 nm [92]
LPEI	~ 50 nm [61]
Poly-L-lysine	25 – 50 nm [91]
Polyethylene glycol-grafted-poly-L-lysine	< 20 nm [96]
Protamine	22 nm [93]
Tetradecane–cysteine–ornithine	35 nm [67]

shown that the endocytosed nanoparticles are colocalized with lysosomes, which suggests that they are not likely to become exocytosed but are potentially available for release into the cytosol. Studies have shown that LTP nanoparticles are endocytosed by primary human dermal fibroblasts. Confocal microscopy has shown that LTP nanoparticles are internalized by fibroblasts. As the result of LTP nanopartices' sizes, which ranges from 100 to 700 nm, endocytosis is the likely means of internalization, but the exact mechanism has yet to be investigated [4]. The participation of other internalization processes, such as pinocytosis, is unlikely because LTP nanoparticles are too large.

5.2 Endosomal escape

Regardless of the internalization and trafficking pathways, the nanoparticles must escape the cellular organelles and enter the cytoplasm in order to provide effective transfection. Failure to escape these organelles results in transfection-incompetent nanoparticles through lysosomal degradation or vesicle entrapment because both are significant barriers to transfection. Similar to the influence of the size and charge upon the internalization and trafficking, the nanoparticle composition also affects endosomal escape. Specifically, the surface charge reversal under acidic conditions [41] and endosomal lysis ('proton-sponge effect') [77] have been attributed as being the mechanisms for nanoparticle release from endolysosomes. Therefore, incorporating materials capable of buffering pH and providing a means of endosomal escape form an important strategy for designing nanoparticles for gene delivery (LPEI incorporation within LTP nanoparticles). Apart from key properties of nanoparticles, cellular phenotype has also been shown to have an effect on escape capabilities of nanoparticles. Ideally, once the nanoparticles are released into the cytoplasm, they must begin to release their genetic material and be transported to the nucleus.

5.3 Nuclear localization signals

The main barrier to non-viral gene delivery is the restriction by the nuclear membrane to prevent the cytoplasmic diffusion of pDNA [78,79]. For fast dividing cells in culture, this event is believed to be the key for transfection during mitosis [80,81]. Thus, free pDNA that is in close proximity could become incidentally sequestered in the nuclei of the daughter cells during telophase. This process would explain why most transfected cells appear as doublets [82]. For slowly growing cells, pDNA can enter through the nuclear pores by diffusion or with carriers. However, the mechanisms of nuclear entry for DNA complexes and for DNA conjugated with nuclear location signal (NLS) are poorly understood [83].

The nuclear pore is a dynamic complex that disassembles before mitosis and reassembles after cellular division. It is a tripartite structure consisting of cytoplasmic and nucleoplasmic rings connected by two sets of eight spokes (the spoke–ring complex) [84-86]. This general structure of the nuclear pore complex seems to be conserved among the eukaryotic cells [86]. The spoke–ring complex forms a central channel with a functional diameter of ~ 26 nm and contains the central transporter [87]. It also has 8 smaller channels that are 9 – 12 nm in diameter [87-89]. Although the roles of these channels are still the subject of debate, larger macromolecules are generally transported through the central transporter by means of carriers such as NLS [87,90]. The smaller channels are thought to allow passive diffusion between the nucleus and cytoplasm [87,90]. Thus, most pDNA condensation methods (**Table 3**) will not theoretically allow for the diffusion of polyplexes or lipoplexes into the nucleus [67,91,92]. However, polyplexes of polyethylenimine and polyethylene glycol-grafted poly-L-lysine (PEG-*g*-PLL) have been reported to gain entry, through an unknown mechanism, into the nuclei of mammalian cells for both *in vitro* and *in vivo* conditions [93-96].

For non-viral gene delivery systems, the use of NLS could be a reliable method for transporting pDNA to the nucleus; however, the results reported in the literature have been mixed and are controversial. The conjugation of NLS to linearized pDNA results in a molecule ~ 3 nm in diameter. It has been reported that the use of NLS–DNA conjugates enhances transfection up to 300-fold when compared with just plasmid DNA [97,98]. However, other reports show no transfection enhancement with the NLS [83,99,100]. So far, the use of NLS has given conflicting results and requires better understanding of the mechanism for DNA entry into the nucleus.

6. Conclusions

For the promises of gene therapy as a treatment for various genetic diseases, cancer and cardiovascular disease to be realized, a safer alternative to the current viral vectors must be developed. Researchers have tried to mimic the viruses' ability to cross cellular membranes, escape endosomes, protect genetic material from enzymes, and evade the immune system by developing non-viral vectors, such as liposomes, cationic polyplexes, and degradable polymeric microparticles and nanoparticles. However, degradable nanoparticles are the only non-viral vector that can mimic the size of the virus and be endocytosed by way of receptor-mediated pathways while

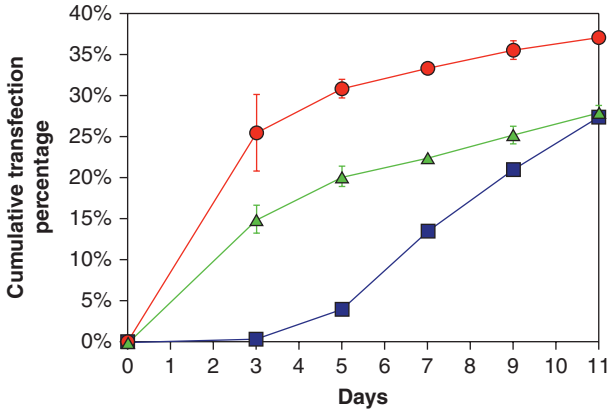

Figure 3. Cumulative cellular transfection of: (●) pDNA–LPEI complex; (▲) pDNA–Fugene6 complex; and (■) LTP nanospheres during a period of 11 days [4].
Reproduced with permission.

Figure 4. Left rat kidney injected with 2.5 mg of l-tyrosine polyphosphate nanoparticles encapsulated with pDNA–LPEI complex (10 µg pDNA by weight). The blue color indicates positive staining for β-galactosidase.

also providing a sustained release of genetic material. Formulating these nanoparticles from a rapidly degrading polymer is optimal for most gene delivery applications, as all the contents of the nanoparticles are delivered before the end of the cell cycle. These nanoparticles can utilize the incorporation of other moieties, such as cationic polymers and PEG, to enhance their transfection capabilities. Furthermore, the surfaces of the nanoparticles can be decorated with targeting molecules for systemic delivery applications.

7. Expert opinion

The authors have stated previously that viral vectors are effective because of their ability to recognize cells, traverse the cellular membrane, escape from endosomes, and transport their genetic material to the nucleus. Retrovirsues also have the ability to evade the immune system. If these functions are replicated within nanoparticles along with their controlled release properties, the authors believe that the weakness of low transfection efficiency for non-viral methods can be surmounted and the promises of gene therapy can be finally realized.

With LTP nanoparticles, the incorporation of LPEI-DNA where LPEI functions to protect pDNA during nanoparticle formulation and for endosomal escape has already been demonstrated. In addition, PEG has been used for both cellular targeting and for evading the immune system. As LTP degrades rapidly, populations of polyplexes and/or free pDNA are released from the nanoparticles into the cytoplasm. **Figure 3** shows the cumulative release profile from LTP nanoparticles. In addition, the authors have shown that LTP nanoparticles are able to transfect primary human dermal fibroblast at a controlled rate. LTP nanoparticles have a delay in transfection for the first 5 days, but the transfection is increased at a linear rate for the next 6 days. At 11 days of transfection, the number of transfected fibroblasts is equal to pDNA-FuGENE 6. In contrast, these lipoplexes and polyplexes (pDNA-LPEI) show maximal transfection at day 3, after which the transfection decreases. Although these results are possibly in part due to the rapid degradation of LTP, similar results could be achieved using other polymers with comparable degradation times.

In addition to controlled transfection of human dermal fibroblasts in an *in vitro* setting, the authors have recently applied LTP-DNA nanoparticles *in vivo*. They have been injected into kidneys and the expression of β-galactosidase has been detected using X-gal staining, which turns transfected tissue green (**Figure 4**). Although this initial result is promising, the *in vivo* evaluation has just begun and requires thorough investigation. Furthermore, the use of a therapeutic gene will be required and applied to an appropriate disease model. Until these studies have been completed, the therapeutic value of the LTP nanoparticles cannot be fully assessed.

The LTP nanoparticles, so far, have been incorporated with many the viral functions. Undoubtedly, the incorporation of a NLS will be a challenge because their results have been conflicting. Regardless, major obstacles will include elucidating the understanding of NLS, generating complexes with NLS peptides that are < 26 nm (sizes of nuclear pores), incorporating the NLS–DNA complex into LTP nanoparticles without altering their sizes, and tracking the release NLS–DNA complexes and their migration into the cell's nucleus. Once this component has been added and verified, the efficacy of the LTP nanoparticle delivery system should

be tested with both dividing and non-dividing cells using viruses as a control. A successful outcome will be the cumulative transfection of the same order of magnitude for both nanoparticles and viral vectors over a 2-week time period.

Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

Bibliography

1. Cristiano RJ. Viral and non-viral vectors for cancer gene therapy. Anticancer Res 1998;18(5A):3241-5
2. Jabr-Milane L, van Vlerken L, Devalapally H, et al. Multi-functional nanocarriers for targeted delivery of drugs and genes. J Control Release 2008;130(2):121-8
3. Ditto AJ, Shah PN, Lopina ST, Yun YH. Nanospheres formulated from L-tyrosine polyphosphate as a potential intracellular delivery device. Int J Pharm 2009;368(1-2):199-206
4. Ditto AJ, Shah PN, Gump LR, Yun YH. Nanospheres formulated from L-tyrosine polyphosphate exhibiting sustained release of polyplexes and in vitro controlled transfection properties. Mol Pharm 2009;6(3):986-95
5. Edelstein ML, Abedi MR, Wixon J, Edelstein RM. Gene therapy clinical trials worldwide 1989-2004-an overview. J Gene Med 2004;6(6):597-602
6. Anderson WF. Human gene therapy. Nature 1998;392(6679 Suppl):25-30
7. Fox JL. Gene therapy safety issues come to fore. Nat Biotechnol 1999;17(12):1153
8. Hollon T. Researchers and regulators reflect on first gene therapy death. Am J Ophthalmol 2000;129(5):701
9. Evans CH, Ghivizzani SC, Robbins PD. Arthritis gene therapy's first death. Arthritis Res Ther 2008;10(3):110
10. Woods NB, Bottero V, Schmidt M, et al. Gene therapy: therapeutic gene causing lymphoma. Nature 2006;440(7088):1123
11. Birnbaum DT, Kosmala JD, Brannon-Pappas L. Optimization of preparation techniques for poly(lactic acid-co-glycolic acid) nanoparticles. J Nanopart Res 2000;2:173-81
12. Olivier JC. Drug transport to brain with targeted nanoparticles. NeuroRx 2005;2(1):108-19
13. Panyam J, Labhasetwar V. Targeting intracellular targets. Curr Drug Deliv 2004;1(3):235-47
14. Stayton PS, Hoffman AS, Murthy N, et al. Molecular engineering of proteins and polymers for targeting and intracellular delivery of therapeutics. J Control Release 2000;65(1-2):203-20
15. Suh H, Jeong B, Rathi R, Kim SW. Regulation of smooth muscle cell proliferation using paclitaxel-loaded poly(ethylene oxide)-poly(lactide/glycolide) nanospheres. J Biomed Mater Res 1998;42(2):331-8
16. De S, Robinson D. Polymer relationships during preparation of chitosan-alginate and poly-l-lysine-alginate nanospheres. J Control Release 2003;89(1):101-12
17. Lu W, Wan J, She Z, Jiang X. Brain delivery property and accelerated blood clearance of cationic albumin conjugated pegylated nanoparticle. J Control Release 2007;118(1):38-53
18. Akinc A, Thomas M, Klibanov AM, Langer R. Exploring polyethylenimine-mediated DNA transfection and the proton sponge hypothesis. J Gene Med 2005;7(5):657-63
19. Gebhart CL, Kabanov A. Perspective on polymeric gene delivery. J Bioactive Compatible Polymers 2003;18(2):147-66
20. Mahato RI, Rolland A, Tomlinson E. Cationic lipid-based gene delivery systems: pharmaceutical perspectives. Pharm Res 1997;14(7):853-9
21. Itaka K, Harada A, Yamasaki Y, et al. In situ single cell observation by fluorescence resonance energy transfer reveals fast intra-cytoplasmic delivery and easy release of plasmid DNA complexed with linear polyethylenimine. J Gene Med 2004;6(1):76-84
22. Fischer D, Bieber T, Li Y, et al. A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: effect of molecular weight on transfection efficiency and cytotoxicity. Pharm Res 1999;16(8):1273-9
23. Ogris M, Steinlein P, Kursa M, et al. The size of DNA/transferrin-PEI complexes is an important factor for gene expression in cultured cells. Gene Ther 1998;5(10):1425-33
24. Ma B, Zhang S, Jiang H, et al. Lipoplex morphologies and their influences on transfection efficiency in gene delivery. J Control Release 2007;123(3):184-94
25. Pouton CW, Seymour LW. Key issues in non-viral gene delivery. Adv Drug Deliv Rev 2001;46(1-3):187-203
26. Watson P, Jones AT, Stephens DJ. Intracellular trafficking pathways and drug delivery: fluorescence imaging of living and fixed cells. Adv Drug Deliv Rev 2005;57(1):43-61
27. Mahato RI, Takakura Y, Hashida M. Nonviral vectors for in vivo gene delivery: physicochemical and pharmacokinetic considerations. Crit Rev Ther Drug Carrier Syst 1997;14(2):133-72
28. Rodriguez A, Flemington EK. Transfection-mediated cell-cycle signaling: considerations for transient transfection-based cell-cycle studies. Anal Biochem 1999;272(2):171-81
29. Kabanov AV, Vinogradov SV, Suzdaltseva YG, Alakhov V. Water-soluble block polycations as carriers for oligonucleotide delivery. Bioconjug Chem 1995;6(6):639-43
30. Wolfert MA, Schacht EH, Toncheva V, et al. Characterization of vectors for gene therapy formed by self-assembly of DNA with synthetic block co-polymers. Hum Gene Ther 1996;7(17):2123-33
31. Nguyen HK, Lemieux P, Vinogradov SV, et al. Evaluation of polyether-polyethyleneimine graft copolymers as gene transfer agents. Gene Ther 2000;7(2):126-38
32. Lee RJ, Huang L. Folate-targeted, anionic liposome-entrapped polylysine-condensed DNA for tumor cell-specific gene transfer. J Biol Chem 1996;271(14):8481-7
33. Duzgunes N, Felgner PL. Intracellular delivery of nucleic acids and transcription factors by cationic liposomes. Methods Enzymol 1993;221:303-6
34. Friend DS, Papahadjopoulos D, Debs RJ. Endocytosis and intracellular processing accompanying transfection mediated by

cationic liposomes. Biochim Biophys Acta 1996;1278(1):41-50

35. Lutz J, Augustin AJ, Jager LJ, et al. Acute toxicity and depression of phagocytosis in vivo by liposomes: influence of lysophosphatidylcholine. Life Sci 1995;56(2):99-106
36. Smyth Templeton N. Liposomal delivery of nucleic acids in vivo. DNA Cell Biol 2002;21(12):857-67
37. Wang CY, Huang L. pH-sensitive immunoliposomes mediate target-cell-specific delivery and controlled expression of a foreign gene in mouse. Proc Natl Acad Sci USA 1987;84(22):7851-5
38. Desai MP, Labhasetwar V, Walter E, et al. The mechanism of uptake of biodegradable microparticles in Caco-2 cells is size dependent. Pharm Res 1997;14(11):1568-73
39. van der Lubben IM, Verhoef JC, van Aelst AC, et al. Chitosan microparticles for oral vaccination: preparation, characterization and preliminary in vivo uptake studies in murine Peyer's patches. Biomaterials 2001;22(7):687-94
40. Chu CJ, Dijkstra J, Lai MZ, et al. Efficiency of cytoplasmic delivery by pH-sensitive liposomes to cells in culture. Pharm Res 1990;7(8):824-34
41. Panyam J, Labhasetwar V. Sustained cytoplasmic delivery of drugs with intracellular receptors using biodegradable nanoparticles. Mol Pharm 2004;1(1):77-84
42. Vasir JK, Labhasetwar V. Biodegradable nanoparticles for cytosolic delivery of therapeutics. Adv Drug Deliv Rev 2007;59(8):718-28
43. Xu ZP, Zeng QH, Lu GQ, Yu AB. Inorganic nanoparticles as carriers for efficient cellular delivery. Chem Eng Sci 2006;61:1027-40
44. Jalil R, Nixon JR. Biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. J Microencapsul 1990;7(3):297-325
45. Desai TR, Hancock RE, Finlay WH. A facile method of delivery of liposomes by nebulization. J Control Release 2002;84(1-2):69-78
46. Tice TR, Tabibi ES. Parenteral drug delivery: injectables. In: Kydonieus AF, editor, Treatise on controlled drug delivery: fundamentals, optimization, applications. New York: Marcel Dekker; 1991. p. 315-39
47. Heller J. Controlled release of biologically active compounds from bioerodible polymers. Biomaterials 1980;1(1):51-7
48. Wu XS. Preparation, characterization and drug delivery applications of microspheres based on biodegradable lactic/glycolic acid polymers. In: Wise DL, editor, Encyclopedic handbook of biomaterials and bioengineering. New York: Marcel Dekker; 1995. p. 1151-200
49. Lewis DH. Controlled release of bioactive agents from lactide/glycolide polymers. In: Chasin M, Langer RS, editors, Biodegradable polymers as drug delivery systems. New York: Marcel Dekker; 1990. p. 1-41
50. Cai Q, Shi G, Bei J, Wang S. Enzymatic degradation behavior and mechanism of poly(lactide-co-glycolide) foams by trypsin. Biomaterials 2003;24(4):629-38
51. Hong Z, Zhang P, He C, et al. Nano-composite of poly(L-lactide) and surface grafted hydroxyapatite: mechanical properties and biocompatibility. Biomaterials 2005;26(32):6296-304
52. Zweers ML, Engbers GH, Grijpma DW, Feijen J. In vitro degradation of nanoparticles prepared from polymers based on DL-lactide, glycolide and poly(ethylene oxide). J Control Release 2004;100(3):347-56
53. Chen X, Ooi CP, Lim TH. Effect of ganciclovir on the hydrolytic degradation of poly(lactide-co-glycolide) microspheres. J Biomater Appl 2006;20(3):287-302
54. Duvvuri S, Janoria KG, Mitra AK. Development of a novel formulation containing poly(d,l-lactide-co-glycolide) microspheres dispersed in PLGA-PEG-PLGA gel for sustained delivery of ganciclovir. J Control Release 2005;108(2-3):282-93
55. Jeong JH, Park TG. Poly(L-lysine)-g-poly(D,L-lactic-co-glycolic acid) micelles for low cytotoxic biodegradable gene delivery carriers. J Control Release 2002;82(1):159-66
56. Gupta AS, Lopina ST. Properties of L-tyrosine based polyphosphates pertinent to biomedical applications. Polymer 2005;46:2133-40
57. Witt C, Mader K, Kissel T. The degradation, swelling and erosion properties of biodegradable implants prepared by extrusion or compression moulding of poly(lactide-co-glycolide) and ABA triblock copolymers. Biomaterials 2000;21(9):931-8
58. Ara M, Watanabe M, Imai Y. Effect of blending calcium compounds on hydrolytic degradation of poly(DL-lactic acid-co-glycolic acid). Biomaterials 2002;23(12):2479-83
59. Gupta AS, Lopina ST. L-tyrosine-based backbone-modified poly(amino acids). J Biomater Sci Polym Ed 2002;13(10):1093-104
60. Ditto A. DNA-LPEI complexes encapsulation in LTP nanospheres as a non-viral gene therapy vector. Akron: The University of Akron; 2006
61. Du H, Chandaroy P, Hui SW. Grafted poly-(ethylene glycol) on lipid surfaces inhibits protein adsorption and cell adhesion. Biochim Biophys Acta 1997;1326(2):236-48
62. Peracchia MT, Fattal E, Desmaele D, et al. Stealth PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting. J Control Release 1999;60(1):121-8
63. Photos PJ, Bacakova L, Discher B, et al. Polymer vesicles in vivo: correlations with PEG molecular weight. J Control Release 2003;90(3):323-34
64. Beletsi A, Panagi Z, Avgoustakis K. Biodistribution properties of nanoparticles based on mixtures of PLGA with PLGA-PEG diblock copolymers. Int J Pharm 2005;298(1):233-41
65. Gref R, Minamitake Y, Peracchia MT, et al. Biodegradable long-circulating polymeric nanospheres. Science 1994;263(5153):1600-3
66. Jeong B, Bae YH, Lee DS, Kim SW. Biodegradable block copolymers as injectable drug-delivery systems. Nature 1997;388(6645):860-2
67. Suh W, Han SO, Yu L, Kim SW. An angiogenic, endothelial-cell-targeted polymeric gene carrier. Mol Ther 2002;6(5):664-72
68. Yun YH, Goetz DJ, Yellen P, Chen W. Hyaluronan microspheres for sustained gene delivery and site-specific targeting. Biomaterials 2004;25(1):147-57

69. Dickerson JB, Blackwell JE, Ou JJ, et al. Limited adhesion of biodegradable microspheres to E- and P-selectin under flow. Biotechnol Bioeng 2001;73(6):500-9

70. Rejman J, Oberle V, Zuhorn IS, Hoekstra D. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochem J 2004;377(Pt 1):159-69

71. Johannes L, Lamaze C. Clathrin-dependent or not: is it still the question? Traffic 2002;3(7):443-51

72. Goncalves C, Mennesson E, Fuchs R, et al. Macropinocytosis of polyplexes and recycling of plasmid via the clathrin-dependent pathway impair the transfection efficiency of human hepatocarcinoma cells. Mol Ther 2004;10(2):373-85

73. Akita H, Ito R, Khalil IA, et al. Quantitative three-dimensional analysis of the intracellular trafficking of plasmid DNA transfected by a nonviral gene delivery system using confocal laser scanning microscopy. Mol Ther 2004;9(3):443-51

74. Medina-Kauwe LK. Endocytosis of adenovirus and adenovirus capsid proteins. Adv Drug Deliv Rev 2003;55(11):1485-96

75. Colin M, Maurice M, Trugnan G, et al. Cell delivery, intracellular trafficking and expression of an integrin-mediated gene transfer vector in tracheal epithelial cells. Gene Ther 2000;7(2):139-52

76. Patil Y, Panyam J. Polymeric nanoparticles for siRNA delivery and gene silencing. Int J Pharm 2009;367(1-2):195-203

77. Kamiya H, Tsuchiya H, Yamazaki J, Harashima H. Intracellular trafficking and transgene expression of viral and non-viral gene vectors. Adv Drug Deliv Rev 2001;52(3):153-64

78. Zabner J, Fasbender AJ, Moninger T, et al. Cellular and molecular barriers to gene transfer by a cationic lipid. J Biol Chem 1995;270(32):18997-9007

79. Labat-Moleur F, Steffan AM, Brisson C, et al. An electron microscopy study into the mechanism of gene transfer with lipopolyamines. Gene Ther 1996;3(11):1010-7

80. Andreadis S, Fuller AO, Palsson BO. Cell cycle dependence of retroviral transduction: an issue of overlapping time scales. Biotechnol Bioeng 1998;58(2-3):272-81

81. Brunner S, Sauer T, Carotta S, et al. Cell cycle dependence of gene transfer by lipoplex, polyplex and recombinant adenovirus. Gene Ther 2000;7(5):401-7

82. Zuber G, Dauty E, Nothisen M, et al. Towards synthetic viruses. Adv Drug Deliv Rev 2001;52(3):245-53

83. van der Aa MA, Koning GA, d'Oliveira C, et al. An NLS peptide covalently linked to linear DNA does not enhance transfection efficiency of cationic polymer based gene delivery systems. J Gene Med 2005;7(2):208-17

84. Akey CW, Radermacher M. Architecture of the Xenopus nuclear pore complex revealed by three-dimensional cryo-electron microscopy. J Cell Biol 1993;122(1):1-19

85. Goldberg MW, Allen TD. Structural and functional organization of the nuclear envelope. Curr Opin Cell Biol 1995;7(3):301-9

86. Heese-Peck A, Raikhel NV. The nuclear pore complex. Plant Mol Biol 1998;38(1-2):145-62

87. Hinshaw JE, Carragher BO, Milligan RA. Architecture and design of the nuclear pore complex. Cell 1992;69(7):1133-41

88. Feldherr CM. The effect of the electron-opaque pore material on exchanges through the nuclear annuli. J Cell Biol 1965;25:43-53

89. Peters R. Nucleo-cytoplasmic flux and intracellular mobility in single hepatocytes measured by fluorescence microphotolysis. EMBO J 1984;3(8):1831-6

90. Talcott B, Moore MS. Getting across the nuclear pore complex. Trends Cell Biol 1999;9(8):312-8

91. Cartier R, Reszka R. Utilization of synthetic peptides containing nuclear localization signals for nonviral gene transfer systems. Gene Ther 2002;9(3):157-67

92. Liu G, Li D, Pasumarthy MK, et al. Nanoparticles of compacted DNA transfect postmitotic cells. J Biol Chem 2003;278(35):32578-86

93. VandeVondele S, Voros J, Hubbell JA. RGD-grafted poly-L-lysine-graft-(polyethylene glycol) copolymers block non-specific protein adsorption while promoting cell adhesion. Biotechnol Bioeng 2003;82(7):784-90

94. Pollard H, Remy JS, Loussouarn G, et al. Polyethylenimine but not cationic lipids promotes transgene delivery to the nucleus in mammalian cells. J Biol Chem 1998;273(13):7507-11

95. Gautam A, Densmore CL, Golunski E, et al. Transgene expression in mouse airway epithelium by aerosol gene therapy with PEI-DNA complexes. Mol Ther 2001;3(4):551-6

96. Ziady AG, Gedeon CR, Miller T, et al. Transfection of airway epithelium by stable PEGylated poly-L-lysine DNA nanoparticles in vivo. Mol Ther 2003;8(6):936-47

97. Zanta MA, Belguise-Valladier P, Behr JP. Gene delivery: a single nuclear localization signal peptide is sufficient to carry DNA to the cell nucleus. Proc Natl Acad Sci USA 1999;96(1):91-6

98. Branden LJ, Mohamed AJ, Smith CI. A peptide nucleic acid-nuclear localization signal fusion that mediates nuclear transport of DNA. Nat Biotechnol 1999;17(8):784-7

99. Nagasaki T, Myohoji T, Tachibana T, et al. Can nuclear localization signals enhance nuclear localization of plasmid DNA? Bioconjug Chem 2003;14(2):282-6

100. Tanimoto M, Kamiya H, Minakawa N, et al. No enhancement of nuclear entry by direct conjugation of a nuclear localization signal peptide to linearized DNA. Bioconjug Chem 2003;14(6):1197-202

Affiliation

Andrew J Ditto[1], Parth N Shah[2] & Yang H Yun[†1]
[†]Author for correspondence
[1]The University of Akron,
Sidney Olson Research Center,
Department of Biomedical Engineering,
Akron, OH 44325, USA
Tel: +1 330 972 6619; Fax: +1 330 374 8834;
E-mail: yy@uakron.edu
[2]The University of Akron,
Whitby Hall,
Department of Chemical and
Biomolecular Engineering,
Akron, OH 44325, USA